

RESPONSE TO RESTRICTION REQUIREMENT
U.S. Appln. No. 10/533,166 (Q87648)

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application:

LISTING OF CLAIMS:

Claim 1. (Currently Amended) An assay method for detecting fungal infection of soil or vegetables by a pathogenic fungal *Pythium* species, ~~in particular *M. acerina*, *F. carotae* and *Pythium* species~~, said method comprising:

~~obtaining a sample of soil or vegetable;~~

(A) treating said a soil or vegetable sample to lyse fungal cells therein;

(B) carrying out~~using an oligonucleotide primer pair,~~ effecting a polymerase chain reaction on DNA released by lysis of the fungal cells, using an oligonucleotide primer pair; and

(C) detecting DNA fragments generated by said polymerase chain reaction so as to detect said fungal infection;

wherein said primer pair comprises an 18- to 24-mer ~~having the ability to hybridize~~ which hybridizes to ~~anone of the~~ oligonucleotide sequences selected from the group consisting of formulae ~~Ia (SEQ ID NO:1), Ib (SEQ ID NO:2), IIa (SEQ ID NO:3), IIb (SEQ ID NO:4), IIIa (SEQ ID NO:5), IIIb (SEQ ID NO:6), IVa (SEQ ID NO:7), IVb (SEQ ID NO:8), Va (SEQ ID NO:9), Vb (SEQ ID NO:10), VIa (SEQ ID NO:11), VIb (SEQ ID NO:12), VIIa (SEQ ID NO:13), VIIb (SEQ ID NO:14), VIIIa (SEQ ID NO:15), VIIIb (SEQ ID NO:16), IXa (SEQ ID NO:17), IXb (SEQ ID NO:18), Xa (SEQ ID NO:19), and Xb (SEQ ID NO:20), XIa (SEQ ID NO:21), XIIb (SEQ ID~~

RESPONSE TO RESTRICTION REQUIREMENT
U.S. Appln. No. 10/533,166 (Q87648)

NO:22), ~~XIIa (SEQ ID NO:23), XIIb (SEQ ID NO:24), XIIIa (SEQ ID NO:25), XIIIb (SEQ ID NO:26), XIVa (SEQ ID NO:27) and XIVb (SEQ ID NO:28):~~

~~5' TCA CTT GTG GCG TAA ACA ACA 3' (Ia)~~
5' - AGA CCA CAA TAA AGC GGC - 3' (Ib)
~~5' AGT CCC GCA CAC ACA CAT 3' (IIa)~~
5' - ACT TCT CTC TTT GGG GAG TGG - 3' (IIb)
~~5' TTC GTT CAG CCT CTG CAT 3' (IIIa)~~
5' - TCG TTT CGG CTA TGA ATA CAG - 3' (IIIb)
5' - ACA AAT ATA CCA ACC ACA GCG - 3' (IVa)
5' - TTT GTA CTT GTG CAA TTG GC - 3' (IVb)
5' - AAC GAA TAT ACC AAC CGC TG - 3' (Va)
5' - TCA TCT ATT TGT GCA CTT CTT TTT - 3' (Vb)
~~5' TCT TCT TTA CCC CAC AAG TGA 3' (VIa)~~
5' - GCC GCT TTA TTG TGG TCT - 3' (VIb)
~~5' ATG TGT CTG TGC GCG ACT 3' (VIIa)~~
5' - CCA CTC CCC AAA GAG AGA AGT - 3' (VIIb)
~~5' ATG CAG AGC CTG AAC GAA 3' (VIIIa)~~
5' - CTG TAT TCA TAG CCG AAA CGA - 3' (VIIIb)
5' - CGC TGT GGT TGG TAT ATT TGT - 3' (IXa)
5' - GCC AAT TGC ACA AGT ACA AA - 3' (IXb)
5' - CAG CGG TTG GTA TAT TCG TT - 3' (Xa)
5' - AAA AAG AAG TGC ACA AAT AGA TGA - 3' (Xb)
~~5' GTT TCA ATG GAG TCC GAC CG 3' (XIa)~~
~~5' CGC CGT ACT TGC TTC GGA GC 3' (XIb)~~
~~5' TGC GAT TAA CGG GCA GAG AC 3' (XIIa)~~
~~5' TTT CGC ATT CGG AGC CTT CG 3' (XIIb)~~
~~5' CGG TCG GAC TCC ATT CAA AC 3' (XIIIa)~~
~~5' GCT CCG AAG CAA GTA CGC CG 3' (XIIIb)~~

RESPONSE TO RESTRICTION REQUIREMENT
U.S. Appln. No. 10/533,166 (Q87648)

~~5' - CTC TCT GCC CGT TAA TCC CA - 3' (XIVa)~~

~~5' - CCA AGC CTC CGA ATG CGA AA - 3' (XIVb).~~

Claims 2-3. (Cancelled).

Claim 4. (Currently Amended) A The method as claimed in claim 12, wherein said primer pair comprises a pair of 18- to 24-mers ~~having the ability which to~~ hybridize to a pair of the oligonucleotide sequences selected from the group consisting of formulae Ia (SEQ ID NO:1) and Ib, ~~or~~ IIA (SEQ ID NO:3) and IIB, ~~or~~ IIIa (SEQ ID NO:5) and IIIB, ~~or~~ IVa and IVb, ~~or~~ and Va and Vb

5' - TCA CTT GTG GGG TAA AGA AGA - 3' (Ia)

5' - AGT CCC GCA CAC ACA CAT - 3' (IIa)

5' - TTC GTT CAG CCT CTG CAT - 3' (IIIa).

Claim 5. (Cancelled).

Claim 6. (Currently Amended) An assay method for detecting fungal infection of soil or vegetables by pathogenic ~~fungal~~ Pythium species, ~~in particular M. acerina, F. carotae and Pythium~~ species, said method comprising:

~~obtaining a sample of soil or vegetable;~~

(A) treating said a soil or vegetable sample to lyse fungal cells therein;

(B) carrying out using an oligonucleotide primer pair, effecting a polymerase chain reaction on DNA released by lysis of the fungal cells, using an oligonucleotide primer pair;

(C) contacting the DNA fragments generated by said polymerase chain reaction with a substrate having immobilized thereon a primer which comprises an 18- to 24-mer having the ability to hybridize which hybridizes to one of the an oligonucleotide

RESPONSE TO RESTRICTION REQUIREMENT
U.S. Appln. No. 10/533,166 (Q87648)

sequences selected from the group consisting of
formulae Ia, Ib, ~~IIa, IIb, IIIa, IIIb~~, IVa, IVb,
Va, Vb, ~~VIa, VIb, VIIa, VIIb, VIIIa, VIIIb~~, IXa,
IXb, Xa, and Xb, ~~XIa, XIb, XIIa, XIIb, XIIIa,~~
~~XIIb, XIVa and XIVb~~:

5' - TCA CTT GTG GGC TAA AGA AGA - 3'	(Ia)
5' - AGA CCA CAA TAA AGC GGC - 3'	(Ib)
5' - AGT CCC GCA CAC ACA CAT - 3'	(IIa)
5' - ACT TCT CTC TTT GGG GAG TGG - 3'	(IIb)
5' - TTC GTT CAG CCT CTG CAT - 3'	(IIIa)
5' - TCG TTT CGG CTA TGA ATA CAG - 3'	(IIIb)
5' - ACA AAT ATA CCA ACC ACA GCG - 3'	(IVa)
5' - TTT GTA CTT GTG CAA TTG GC - 3'	(IVb)
5' - AAC GAA TAT ACC AAC CGC TG - 3'	(Va)
5' - TCA TCT ATT TGT GCA CTT CTT TTT - 3'	(Vb)
5' - TCT TCT TTA CCC CAC AAG TGA - 3'	(VIa)
5' - GCC GCT TTA TTG TGG TCT - 3'	(VIb)
5' - ATG TGT GTG TGC GGG ACT - 3'	(VIIa)
5' - CCA CTC CCC AAA GAG AGA AGT - 3'	(VIIb)
5' - ATG CAG AGG CTG AAC GAA - 3'	(VIIIa)
5' - CTG TAT TCA TAG CCG AAA CGA - 3'	(VIIIb)
5' - CGC TGT GGT TGG TAT ATT TGT - 3'	(IXa)
5' - GCC AAT TGC ACA AGT ACA AA - 3'	(IXb)
5' - CAG CGG TTG GTA TAT TCG TT - 3'	(Xa)
5' - AAA AAG AAG TGC ACA AAT AGA TGA - 3'	(Xb)
5' - GTT TGA ATG CAG TCC CAC CG - 3'	(XIa)
5' - CGG CGT ACT TGC TTC GCA GC - 3'	(XIb)
5' - TGC CAT TAA CCG GCA CAG AC - 3'	(XIIa)
5' - TTT CGC ATT CGG AGG CTT GG - 3'	(XIIb)

RESPONSE TO RESTRICTION REQUIREMENT
U.S. Appln. No. 10/533,166 (Q87648)

5' ~~CGG TCG CAC TCC ATT CAA AC~~ 3' ~~(XIIIIa)~~
5' ~~GCT CCG AAG CAA GTA CGC CG~~ 3' ~~(XIIIIb)~~
5' ~~CTC TCT GCC CCT TAA TCC CA~~ 3' ~~(XIVa)~~
5' ~~CCA AGC CTC CGA ATG CGA AA~~ 3' ~~(XIVb)~~; and

(D) detecting DNA fragments binding to said primer so
as to detect said fungal infection.

Claim 7. (Currently Amended) An 18- to 24-mer oligonucleotide primer which hybridizes ~~hybridizable~~ to an oligonucleotide sequence selected from ~~those of the group~~ consisting of formulae Ia, Ib, IIa, IIb, IIIa, IIIb, IVa, IVb, Va, Vb, VIa, VIb, VIIa, VIIb, VIIIa, VIIIb, IXa, IXb, Xa, and Xb, XIa, XIb, XIIa, XIIb, XIIIa, XIIIb, XIVa and XIVb.

Claims 8-9. (Cancelled).

Claim 10. (Currently Amended) ~~A~~The primer as claimed in claim 7, wherein said primer comprises a sequence selected from the group consisting of formulae Ia, Ib, IIa, IIb, IIIa, IIIb, IVa, IVb, Va, Vb, VIa, VIb, VIIa, VIIb, VIIIa, VIIIb, IXa, IXb, Xa, and Xb, XIa, XIb, XIIa, XIIb, XIIIa, XIIIb, XIVa or XIVb or a derivative thereof.

Claim 11. (Currently Amended) A substrate having immobilized thereon at least one 18- to 24-mer oligonucleotide primer ~~hybridizable~~ which hybridizes to an oligonucleotide sequence selected from ~~those of the group consisting of~~ formulae ~~Ia, Ib, IIa, IIb, IIIa, IIIb, IVa, IVb, Va, Vb, VIa, VIb, VIIa, VIIb, VIIIa, VIIIb, IXa, IXb, Xa, and Xb, XIa, XIb, XIIa, XIIb, XIIIa, XIIIb, XIVa and XIVb.~~

Claim 12. (Currently Amended) The substrate as claimed in claim 11, wherein said primer comprises a sequence selected from the group consisting of formulae Ia, Ib, IIa, IIb, IIIa, IIIb,

RESPONSE TO RESTRICTION REQUIREMENT
U.S. Appln. No. 10/533,166 (Q87648)

IVa, IVb, Va, Vb, ~~VIa, VIb, VIIa, VIIb, VIIIA, VIIIB, IXa, IXb, Xa, and Xb, XIa, XIb, XIIa, XIIb, XIIIa, XIIIb, XIVA or XIVb or a derivative thereof.~~

Claim 13. (Currently Amended) A primer composition comprising a pair of 18- to 24-mer oligonucleotide primers, at least one of which ~~is hybridizable~~ hybridizes to an oligonucleotide sequence selected from the group consisting of formula Ia, formulae Ib, IIa, IIb, IIIa, IIIb, IVa, IVb, Va, Vb, VIa, VIb, VIIa, VIIb, VIIIA, VIIIB, IXa, IXb, Xa, and Xb, XIa, XIb, XIIa, XIIb, XIIIa, XIIIb, XIVA or XIVb, optionally together with a carrier.

Claim 14. (Currently Amended) ~~A~~The primer composition as claimed in claim 13, wherein at least one of said pair is a primer comprising a sequence selected from the group consisting of formulae Ia, Ib, IIa, IIb, IIIa, IIIb, IVa, IVb, Va, Vb, VIa, VIb, VIIa, VIIb, VIIIA, VIIIB, IXa, IXb, Xa, and Xb, XIa, XIb, XIIa, XIIb, XIIIa, XIIIb, XIVA or XIVb or a derivative thereof.

Claims 15-16. (Cancelled).

Claim 17. (Currently amended) A kit for the ~~performance of the assay method of any one of claims 1 to 5~~ detecting fungal infection of soil or vegetables by a pathogenic *Pythium* species, said kit comprising at least one ~~primer pair as defined in any one of claims 7, 8 or 10 to 5 together with instructions for the performance of the assay method~~ pair of 18- to 24-mer oligonucleotide primers, at least one of which hybridizes to an oligonucleotide sequence selected from the group consisting of formulae Ib, IIb, IIIb, IVa, IVb, Va, Vb, VIb, VIIb, VIIIB, IXa, IXb, Xa, and Xb, together with instructions for the performance of the assay method.

RESPONSE TO RESTRICTION REQUIREMENT
U.S. Appln. No. 10/533,166 (Q87648)

Claims 18-21. (Cancelled).

Claim 22. (New) The kit as claimed in claim 17, wherein at least one of said pair is a primer comprising a sequence selected from the group consisting of formulae Ib, IIb, IIIb, IVa, IVb, Va, Vb, VIb, VIIb, VIIIb, IXa, IXb, Xa, and Xb.